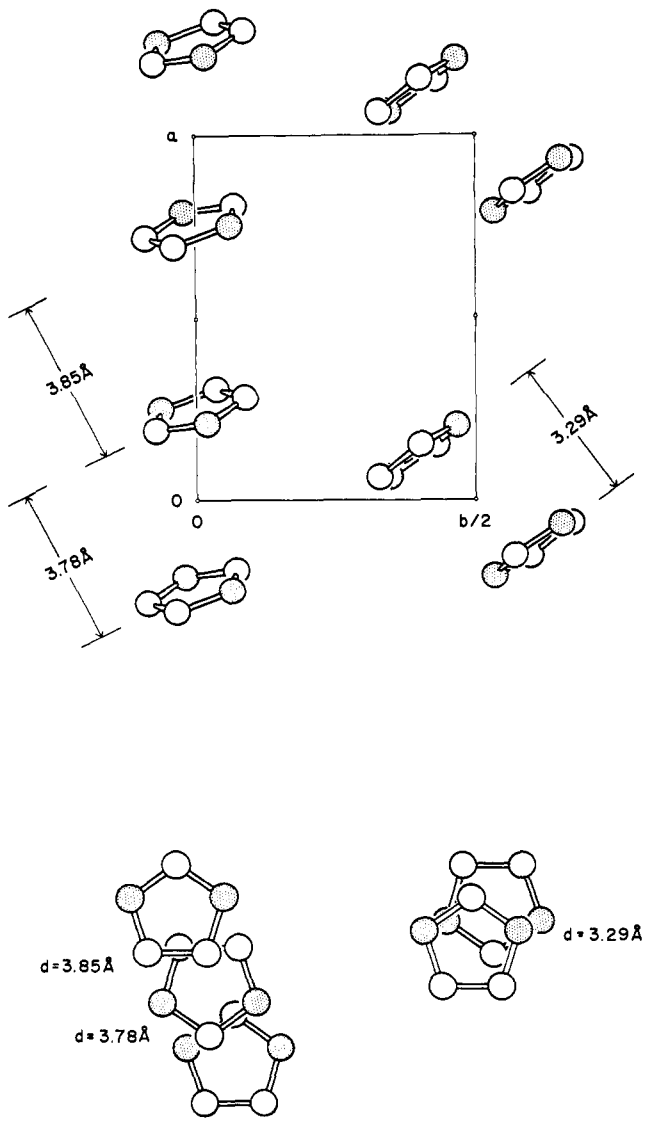

Figure 1. Stacking of the imidazolium rings viewed from the $-c^*$ direction. The stippled spheres represent the nitrogen atoms; hydrogen atoms are not shown. The distances indicated are the perpendicular ring plane to ring plane distances.

A detailed account of the structure analysis and crystal chemistry will be published soon.

In the crystal structure the phosphate anions are linked together by short, strong (P–O–H···O–P) hydrogen bonds to form a three-dimensional network, one feature of which is that there are large channels in the phosphate network along the lines $[x,0,0]$, $[x,{}^1/_2,{}^1/_2]$, $[x,{}^1/_2,0]$, and $[x,0,{}^1/_2]$. The imidazolium cations are stacked in columns filling these channels and are linked to the surrounding phosphate network by (N–H···O–P) hydrogen bonds.

The columns of stacked imidazolium rings are illustrated in Figure 1. Within each column any two adjacent rings are related by a center of symmetry. The degrees of overlapped contact between the stacked rings are shown at the bottom of Figure 1, where centrosymmetrically related pairs of rings are illustrated as viewed in projection along the directions normal to their mean planes. The atoms in each ring are, within experimental error, coplanar.

We observe close agreement between corresponding interatomic distances and angles in the two independent

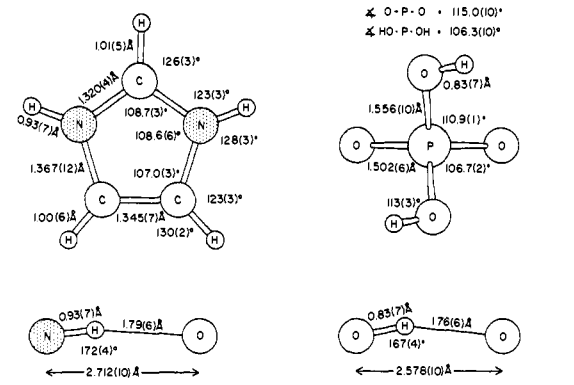

Figure 2. Averaged interatomic distances and angles. The estimated standard deviation given in parentheses is the larger of the two values from (1) the least-squares refinement and (2) the sample averaging (see text).

cations and anions and in the four independent examples of each of the two chemical kinds of hydrogen bonds in the crystallographically asymmetric unit. Moreover, in accord with chemical intuition, the two imidazolium cations very nearly conform to the symmetry of point group $2m$ (C_{2v}); similarly, the two independent tetrahedral phosphate anions are close to point group 2 (C_2). Interatomic distances and angles averaged over this noncrystallographic symmetry as well as over the asymmetric unit are given in Figure 2. When compared with values reported for other (N–H···O) hydrogen bonds,[5] the distances and angles in the hydrogen bonds between the imidazolium rings and phosphate groups in this structure indicate that these hydrogen bonds are among the strongest (N–H···O) bonds yet reported.

(5) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," W. H. Freeman, San Francisco, Calif., 1960, pp 282–285; W. C. Hamilton and J. A. Ibers, "Hydrogen Bonding in Solids," W. A. Benjamin, New York, N. Y., 1968, pp 259–265.

(6) We are grateful for the financial support of this work by the National Institutes of Health (Training Grant No. GM-01728) and by the Health Research and Services Foundation of Pittsburgh (Grant No. L71).

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Enzymatic Synthesis of Cephalosporins

Sir:

Cephalosporins have been synthesized by chemical N-acylation of 7-amino-3-cephem compounds with the corresponding organic acids. For the preparation of cephalosporins bearing a free amino group in the side chain, the amino group of the amino acids employed for N-acylation has usually been blocked by a suitable protecting group before use. For example, cephalexin has been prepared by N-acylation of 7-aminodeacetoxycephalosporanic acid trichloroethyl ester with *N-tert*-butoxycarbonyl-D-α-phenylglycine, followed by stepwise deblocking of the doubly protected form of cephalexin.[1] We wish to report an enzymatic

(1) R. R. Chauvette, P. A. Pennington, C. W. Ryan, R. D. G. Cooper,

Table I. Cephalosporin Synthesis by *Xanthomonas citri* IFO 3835[a]

Expt no.	Organic acid	Substrate concn, mg/ml		Cephalosporin content, mg/ml, in reaction mixture at (min)				
		Acid	7-ADCA	10	20	30	60	90
1	D-Phenylglycine methyl ester	25	10	6.8	10.5	13.9	14.9	14.5
2	L-Phenylglycine methyl ester	25	10	1.8	2.5	3.1	3.7	3.9
3	Glycine ethyl ester	25	10	9.9	12.0	12.1	11.3	10.0
4	D-Alanine ethyl ester	25	10	6.8	10.7	12.0	12.6	12.1
5	D-Leucine ethyl ester	25	10	6.8	9.7	11.2	12.7	12.9
6	D-α-(1-Cyclohexenyl)glycine methyl ester	7.5	2.5	1.9	2.4	2.5	1.9	1.3
7	D-α-(*p*-Hydroxyphenyl)glycine methyl ester	7.5	2.5	1.9	2.5	2.5	2.0	1.2
8	D-α-Cyclohexylglycine methyl ester	7.5	2.5	2.0	2.3	2.2	1.6	0.8
9	β-Alanine methyl ester	25	10	0.0	0.0	0.0	0.0	0.0
10	γ-Aminobutyric acid methyl ester	25	10	0.0	0.0	0.0	0.2	0.0
11	DL-Mandelic acid methyl ester	25	10	0.0	0.0	0.0	0.0	0.1
12	Phenylacetic acid	25	10	0.0	0.0	0.0	0.0	0.0
13	Phenylacetic acid ethyl ester	25	10	0.0	0.1	0.1	0.0	0.0
14	Phenoxyacetic acid methyl ester	25	10	0.0	0.0	0.0	0.0	0.0

[a] The reaction conditions were similar to those described in the text unless otherwise specified. The cephalosporin content was determined by spectrophotometric assay.[11] The cephalosporins thus obtained contain a new compound, 7-[α-amino-α-(1′-cyclohexenyl)acetamido]-3-deacetoxycephalosporanic acid, synthesized in experiment 6, whose physical constants are as follows: mp 185–186° dec; $[\alpha]^{25}D$ +86.2° (*c* 0.5, H_2O); $E^{1\%}_{1\,cm}$ at 260 mμ 215; nmr (δ values in D_2O at pH 3) 1.66 (br, 4 H), 2.10 (br, 4 H), 2.20 (s, 3 H), 3.54 (AB-type quartet, 2 H), 4.63 (s, 1 H), 5.19 (d, 1 H), 5.72 (d, 1 H), and 6.18 (br, 1 H); br, broad; s, singlet; d, doublet.

Table II. Cephalexin and Cephaloglycine Syntheses by Various Bacteria[a]

Bacterial strain	Substrate concn, mg/ml, of D-Phenylglycine methyl ester	Substrate concn, mg/ml, of 7-ADCA or 7-ACA	Accumulation, mg/ml, in reaction mixtures of Cephalexin	Accumulation, mg/ml, in reaction mixtures of Cephaloglycine
Xanthomonas citri IFO 3835	25	10	11.5	7.2
Xanthomonas oryzae IFO 3995	25	10	8.6	6.6
Acetobacter pasteurianus ATCC 6033	25	10	7.4	4.0
Acetobacter turbidans ATCC 9325	25	10	10.3	7.6
Gluconobacter suboxydans ATCC 621	25	10	5.2	5.2
Pseudomonas melanogenum IFO 12020	25	10	7.9	6.5
Mycoplana dimorpha IFO 13213	25	10	7.1	4.0
Protaminobacter alboflavus IFO 13221	25	10	8.0	6.0

[a] The experimental conditions were somewhat different from those described in the text. Thus, the bacterial cells other than those of xanthomonads were grown for 24 hr in a medium containing potato juice (10%), yeast extract (1.0%), thioglycollate culture medium, dehydrated (Daigo Nutritive Chemicals, Ltd., Japan) (1.0%), glycerol (1.5%), and glucose (0.3%) (pH 7). For the preparation of cell suspensions, harvested and washed cells were resuspended in 0.2 *M* phosphate buffer (pH 6.0) at twice the cell density in the growth medium. Reactions were carried out for 60 min without control of pH. The cephalosporin content was determined by spectrophotometric assay[11] and confirmed by conventional bioassay.[12]

synthesis of cephalexin and its analogs from their corresponding organic acid esters and 7-aminocephem compounds in a single step by means of bacteria of the family *Pseudomonadaceae*.

The ability of some microorganisms to synthesize semisynthetic penicillins from 6-aminopenicillanic acid and the corresponding organic acids is well recognized,[2-9] but this is not the case with semisynthetic cephalosporins. To our best knowledge there has been no observation that microorganisms can synthesize semisynthetic cephalosporins from their corresponding organic acids and 7-aminocephem compounds.

We recognized that some bacteria which showed no ability to synthesize penicillin G from phenylacetic acid and 6-aminopenicillanic acid had a strong ability to catalyze N-acylation of 7-amino-3-deacetoxycephalosporanic acid (7-ADCA) with α-amino acid esters. A typical experiment for cephalexin synthesis by *Xanthomonas citri* IFO 3835 was carried out as follows. Five milliliters of the full-growth (16-hr) culture of the bacterium was inoculated into 200 ml of a medium containing sodium glutamate (0.2%), yeast extract (0.2%), peptone (0.5%), dipotassium phosphate (0.2%), magnesium chloride (0.1%), ferrous sulfate (0.01%), and sucrose (2.0%) (pH 7.2). After cultivation for 16 hr at 28° on a rotary shaker the bacterial cells (3×10^9 cells/ml) were separated from the culture by centrifugation, washed with 200 ml of distilled water, and resuspended[10] in 200 ml of distilled water (pH 6.0) containing D-α-phenylglycine methyl ester (5.0 g) and 7-ADCA (2.0 g). The suspension whose pH was maintained at 6.0 by the addition of 2 *N* NaOH through a pH-stat was incubated with stirring for 90 min at 37°, resulting in accumulation of 2.9 g of cephalexin.[11] The suspension

F. L. Jose, I. G. Wright, E. M. Van Heyningen, and G. W. Huffman, *J. Org. Chem.*, **36**, 1259 (1971).
(2) W. Kaufmann and K. Bauer, *Naturwissenschaften*, 20, 474 (1960).
(3) W. Kaufmann, K. Bauer, and H. A. Offe, *Antimicrob. Ag. Annu.*, 1 (1960).
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(5) W. Kaufmann, *Biochim. Biophys. Res. Commun.*, **14**, 458 (1964).
(6) M. Cole, *Biochem. J.*, **115**, 747 (1969).
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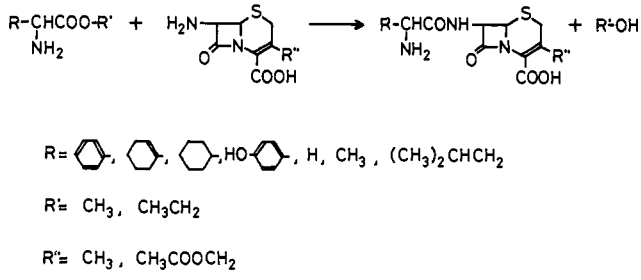
(8) T. Nara, R. Okachi, and M. Misawa, *J. Antibiot.*, **24**, 321 (1971).
(9) T. Nara, M. Misawa, and R. Okachi, *Agr. Biol. Chem.*, **35**, 1676 (1971).
(10) E. F. Gale, *Bacteriol. Rev.*, 7, 139 (1943).
(11) The cephalosporin content in reaction mixtures was determined by a spectrophotometric method involving selective hydrolysis of cephalosporins by cephalosporinase of *Aerobacter cloaceae* IFO 12937 which was found to hydrolyze the β-lactam ring of cephalosporins but not of 7-aminocephem compounds. A difference in absor-

was centrifuged to remove cells and the supernatant fluid thus obtained was chromatographed on an Amberlite XAD-2 column to give 2.2 g of crystals identical in all respects with an authentic sample of cephalexin monohydrate.



In a similar fashion, enzymatic synthesis was also achieved when the D-α-phenylglycine methyl ester was replaced by the esters of other α-amino acids such as glycine, D-alanine, D-leucine, D-α-(1-cyclohexenyl)glycine, D-α-(*p*-hydroxyphenyl)glycine, and D-α-cyclohexylglycine. However, β-alanine, γ-aminobutyric acid, DL-mandelic acid, phenylacetic acid, and phenoxyacetic acid were not substrates for the enzymatic reaction, the results being given in Table I.

Besides xanthomonads, the like synthesizing ability was found among the strains belonging to the family *Pseudomonadaceae* as shown in Table II which indicates that 7-aminocephalosporanic acid (7-ACA) is also a good substrate for the enzymatic reaction.

Acknowledgment. We wish to thank Dr. T. Miki, Takeda Research Laboratories, for his kind supply of some substrate compounds. We are also indebted to Drs. S. Tatsuoka and R. Takeda of our laboratories for their advice and encouragement.

bance at 260 mμ between before and after treatment of a reaction mixture with the enzyme was proportional to a cephalosporin content in the mixture. The results obtained on samples of cephalexin and cephaloglycine showed good agreement with those recorded by the conventional microbiological method.[12] The spectrophotometric assay was applicable to all the cephalosporins described in this communication. The details of the method will be published elsewhere.

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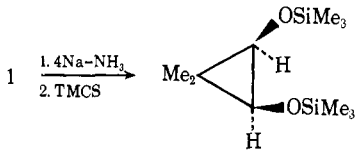
Metal Reduction of Malonates. Formation and Isolation of 3,3-Dimethyl-*cis*-1,2-cyclopropanediol

Sir:

In a recent communication it was recorded that dimethyl dimethylmalonate (**1**) and sodium dispersed in xylene containing trimethylchlorosilane (TMCS) gave dimethylketene methyl trimethylsilyl acetal (**2**) according to the following equation.[1] We now report

$$\underset{\mathbf{1}}{Me_2C(CO_2Me)_2} \xrightarrow[TMCS]{Na\text{–}xylene} Me_2C{=}C(OMe)OSiMe_3 + CO + MeOSiMe_3$$
$$\mathbf{2}$$

that the reduction of **1** with 4 equiv of sodium in liquid ammonia followed by TMCS gave five products including **2** and the cyclopropane ring system 3,3-dimethyl-*cis*-1,2-bistrimethylsilyloxycyclopropane[2] (**3**): bp 30° (0.05 mm); nmr ($CDCl_3$) δ 0.12 (18 H), 0.85



3

(3 H), 0.90 (3 H), 2.75 (2 H); ir ($CHCl_3$) 2980, 1460, 1380, 1350, 1170, 1030 cm^{-1}; mass spectrum *m/e* (rel intensity) 246 (6) M, 73 (100).

Metal reduction of **1** under various conditions formed the products listed in Table I; compounds **4**–**7** are described below.

Compound **4**, $Me_2C(CH_2OSiMe_3)_2$, displayed the following physical properties: bp 30° (0.05 mm); nmr ($CDCl_3$) δ 0.10 (18 H), 0.80 (6 H), 3.30 (4 H); ir ($CHCl_3$) 2985, 1475, 1400, 1360, 1090 (br), 1010 cm^{-1}; mass spectrum *m/e* 233 (4, M − 15), 147 (100). Compound **4** was solvolyzed to 2,2-dimethyl-1,3-propanediol.

Compound **5**, $Me_2C(CH_2OSiMe_3)CONHSiMe_3$, showed the following physical properties: bp 45° (0.05 mm); nmr ($CDCl_3$) δ 0.10 (9 H), 0.20 (9 H), 1.10 (6 H), 3.50 (2 H); ir ($CHCl_3$) 3350, 2980, 1660, 1430 (br), 1080 cm^{-1}; mass spectrum *m/e* 246 (6, M − 15), 73 (100). Compound **5** was hydrolyzed to 2,2-dimethyl-3-hydroxypropionamide, mp 72°.

Compound **6**, $Me_2CHCONHSiMe_3$, displayed the following physical properties: mp 84°; nmr ($CDCl_3$) δ 0.20 (9 H), 1.12 (d, 6 H, J = 6.5 Hz), 2.3 (m, 1 H); ir ($CHCl_3$) 3420, 2980, 1660 (br), 1430 cm^{-1}; mass spectrum *m/e* 159 (6, M), 73 (100). Hydrolysis of **6** gave isobutyramide.

Compound **7**, $Me_2CHCH_2OSiMe_3$, showed the following physical properties: nmr ($CDCl_3$) δ 0.10 (9 H), 0.85 (d, 6 H, J = 12 Hz), 1.7 (m, 1 H), 3.30 (d, 2 H, J = 12 Hz). Solvolysis of **7** gave isobutyl alcohol.

Candidates as intermediates in the reaction of **1** and sodium–liquid ammonia included $Me_2C(CHO)CO_2Me$ (**8**) and $Me_2C(CONH_2)CO_2Me$ (**9**).[3] Addition of the lithium salt of methyl isobutyrate to methyl formate gave **8**: nmr (CCl_4) δ 1.30 (6 H), 3.7 (3 H), 9.6 (1 H); ir ($CHCl_3$) 3000, 2980, 2715, 1725 (br), 1370 cm^{-1}; mass spectrum *m/e* 102 (64, M − 28), 41 (100).

Compound **8** was reduced with sodium–liquid ammonia followed by TMCS and gave products in the relative amounts of 25% **3**, 25% **4**, 5% **6**, and 45% **7**, although more residue than normal remained. Under the same conditions **9** gave only **5** and **6** in a ratio of 4:1. Thus, under these conditions **9** is eliminated as an intermediate and **8** is an unlikely intermediate since it gave **7**.

The intermediacy of a three-membered ring enediol dianion in the formation of **3** does not seem likely.[4]

(1) Y. N. Kuo, F. Chen, C. Ainsworth, and J. J. Bloomfield, *Chem. Commun.*, 136 (1971).

(2) The cis stereochemistry of **3** is established from the fact that the methyl groups have different shift values in the nmr spectrum.

(3) W. H. Perkin, *J. Chem. Soc.*, 83, 1217 (1903).

(4) Reaction of dimethyl succinate with sodium–liquid ammonia at −34° followed by silation using excess TMCS gave 1,2-bistrimethylsilyloxycyclobutene[5] but no 1,2-bistrimethylsilyloxycyclobutane was formed.

(5) J. J. Bloomfield, *Tetrahedron Lett.*, 587 (1968).